

Is Crohn's disease caused by a mycobacterium? Comparisons with leprosy, tuberculosis, and Johne's disease

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Although Crohn's disease is considered to be autoimmune in origin, there is increasing evidence that it may have an infectious cause. The most plausible candidate is *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Intriguingly, Koch's postulates may have been fulfilled for MAP and Crohn's disease, even though they still have not been met for *Mycobacterium leprae* and leprosy. In animals MAP causes Johne's disease, a chronic wasting intestinal diarrhoeal disease evocative of Crohn's disease. Johne's disease occurs in wild and domesticated animals, including dairy herds. Viable MAP is found in human and cow milk, and is not reliably killed by standard pasteurisation. MAP is ubiquitous in the environment including in potable water. Since cell-wall-deficient MAP usually cannot be identified by Ziehl-Neelsen staining, identification of MAP in human beings requires culture or detection of MAP DNA or RNA. If infectious in origin, Crohn's disease should be curable with appropriate antibiotics. Many studies that argue against a causative role for MAP in Crohn's disease have used antibiotics that are inactive against MAP. However, trials that include macrolide antibiotics indicate that a cure for Crohn's disease is possible. The necessary length of therapy remains to be determined. Mycobacterial diseases have protean clinical manifestations, as does Crohn's disease. The necessity of stratifying Crohn's disease into two clinical manifestations (perforating and non-perforating) when interpreting the results of antibiotic therapy is discussed. Rational studies to evaluate appropriate therapies to cure Crohn's disease are proposed.

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Crohn's disease^{1,2} is a disease of unknown aetiology that is increasing in incidence in the USA,^{3–5} Canada,⁶ and around the world.^{7–9} It is associated with immune dysregulation.^{10–12} I review the increasing evidence that Crohn's disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP).

The thesis that Crohn's disease is infectious, and may be caused by MAP, is usually discounted or even ignored.¹³ There are multiple reasons for this scepticism. One is that mycobacteria are not seen using standard mycobacterial cell wall (Ziehl-Neelsen¹⁴) staining techniques. An additional and more compelling concern is that in most antibiotic clinical trials, Crohn's disease has not been cured. The apparent value of immune modulation and the identification of a "Crohn's-related gene" are additional reasons to question bacterial culpability. Despite this

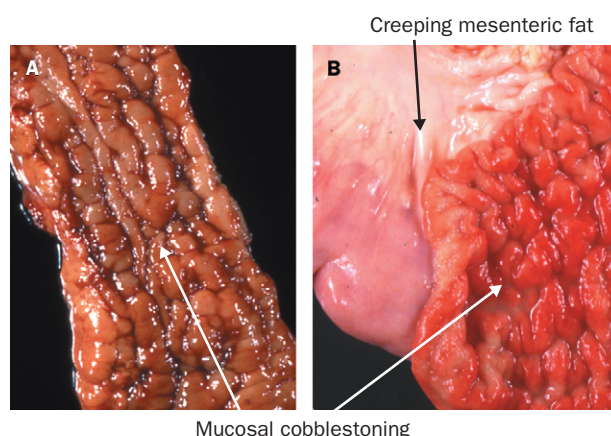


Figure 1. Mucosal cobblestoning in bovine Johne's disease (B; courtesy A J Cooley, School of Veterinary Medicine, University of Wisconsin) compared with that seen in Crohn's disease (A). Additionally, note the creeping mesenteric fat, that is so characteristic of Crohn's disease, on the serosal surface of the specimen from Johnes's disease (arrow).

ongoing controversy, several European governments are concerned about^{15–17} and are addressing the possibility of a causal connection between MAP and Crohn's disease.^{18,19} The government of the UK has decided to exercise the precautionary principle concerning a possible link between Crohn's disease and MAP, and has decided to eradicate MAP from the food chain. This action is not contingent on demonstrating an unequivocal link between MAP and Crohn's disease.¹⁹

Mycobacteria are effective pathogens

Mycobacterium tuberculosis infects about one-third of humankind today.²⁰ However, only an estimated 5–10% of those exposed have a lifetime risk of developing active tuberculosis.²¹ An even smaller minority of those who are infected are killed by the disease.²² Nevertheless, *M tuberculosis* has killed about one billion people over the past two centuries.²³ I suggest that MAP is an equally effective and even more insidious pathogen than *M tuberculosis*. It infects many species and yet, in human beings, is more difficult to detect than *M tuberculosis*.

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MAP in the environment and in animals

MAP is found in the potable water supply of large cities in industrialised nations.²⁴ Mycobacteria are at least two orders of magnitude more resistant to chlorine purification than *Escherichia coli*.²⁵ MAP survives higher concentrations of chlorine (two parts per million) than the 1.1 parts per million routinely achieved with first-use municipal water in the USA.²⁶ Additionally, mycobacteria are more resistant to chlorine purification at the low-nutrient, low-temperature, and increased-pH conditions²⁵ that may be encountered in water systems.

In animals, MAP causes Johne's disease,²⁷ a chronic wasting diarrhoeal intestinal disease that is highly evocative of Crohn's disease (figure 1). Johne's disease is found among primates,²⁸ wild rabbits,²⁹ 18 non-ruminant wildlife species in Scotland,³⁰ free-ranging bighorn sheep and Rocky Mountain goats,³¹ bison,³² wild antelopes imported to a zoo in the USA,³³ and deer in the Czech Republic.³⁴ MAP was cultured from 20% of apparently healthy dwarf goats in a zoo³⁵ where they were available for youngsters to pet.

MAP damages the agricultural industry on several continents.^{36–38} As many as 58% of dairy herds in a given geographical area may be infected.³⁹ The resulting financial damage of Johne's disease is enormous. In the USA, the estimated cost of Johne's disease in 1998 was \$1.5 billion.³⁷

MAP in milk

MAP in milk from domestic animals is well described,^{40–44} and it is not reproducibly eradicated by pasteurisation at the parameters routinely used in the USA.^{15,45–49} There are concerns that using pasteurisation standards effective against MAP may adversely effect the taste and, therefore, consumer acceptance of milk products.¹⁵

MAP has been cultured from the milk of two women with active Crohn's disease who were breast feeding.⁵⁰ Identification was confirmed by the characteristics of the cultured mycobacteria and by detection of the IS900 DNA insertion sequence that is typical of MAP.⁵¹

Pathogenicity of MAP in human beings

The dogma that MAP is not a zoonotic organism—ie, that it is not pathogenic in human beings—is challenged by two case reports. A 36-year-old patient with haemophilia, AIDS, and a CD4+ lymphocyte count of $29 \times 10^6/\text{mL}$ developed vicious diarrhoea. Acid-fast bacilli were seen in biopsy samples from his colon, liver, and bone marrow. Uniquely in human beings, these cell-wall-containing mycobacteria were identified as MAP by both culture (requiring mycobactin containing medium) and IS900 DNA analysis.^{52,53} In a second report, MAP was cultured from draining lymph nodes in the neck of a 6-year-old boy. 5 years later he developed typical ileocaecal Crohn's disease.⁵⁴

Ziehl-Neelsen staining for MAP

Both of the publications that identified Crohn's disease^{1,2} commented on a major difference between the disease they were describing and intestinal tuberculosis: a lack of identifiable acid-alcohol-fast-staining bacteria by the technique described by Ziehl and Neelsen in 1886.⁵⁵ This

staining method identifies the complex and characteristic cell wall of intact mycobacteria. The major difference between Crohn's disease and Johne's disease is that the causative organism can be seen in some animals with Johne's disease.

If the MAP present is the cell-wall-deficient form, the Ziehl-Neelsen stain will be negative. By contrast with cows, in species such as sheep, it is the paucibacillary form of MAP that predominates. In specimens obtained from sheep, prolonged incubation may be required to grow the organism, suggesting that MAP may be present in the cell-wall-deficient form. These protoplasts or "L form" bacteria are Ziehl-Neelsen-negative. Under appropriate culture conditions and over a prolonged period (weeks to years), these protoplasmic bacteria may produce a cell wall,^{56–59} and will then become Ziehl-Neelsen positive.

In human beings, MAP may exist in the cell-wall-deficient form.^{56–61} If this is the case, means of identification such as cell culture,^{62,63} detection of DNA,⁶⁴ RNA,²⁴ or MAP-specific proteins,⁶⁵ or in-situ hybridisation^{66–70} become necessary to confirm the presence of the organism.

Growing MAP in vitro

Part of the problem in diagnosing MAP infections in human beings may be due to the difficulty of growing MAP in vitro.^{60,71} In part the culture problem is due to the fact that MAP lacks the iron-chelating agent mycobactin. As a consequence, either the infected host or the culture medium must provide the MAP with iron for it to grow. Achieving growth may be even more difficult when the organism is initially in its cell-wall-deficient form.^{56,57} Although culturing MAP is difficult, it is not impossible. Indeed, commercial techniques are now available that permit a much more reproducible culture of MAP, at least from animals with Johne's disease.⁷² By comparison, it should be remembered that *Mycobacterium leprae*, universally acknowledged as the cause of leprosy, has not been grown in vitro.⁷³

Isolating the DNA and RNA of MAP

The DNA sequence IS900, first identified in 1989,⁵¹ is considered the "gold standard" to differentiate MAP from other mycobacteria.^{41–43,50–52,54,59,64,66,67,74–80} It must be emphasised that the mere presence of IS900 DNA is not pathognomonic of a causal relation between Crohn's disease and MAP. Importantly, the isolation of DNA of MAP from patients with Crohn's disease is not reproducible. In my laboratory, although we identified the RNA of MAP in 100% of cases,²⁴ we could not reproducibly obtain MAP DNA from the same intestinal tissue using standard (proprietary commercially available) DNA extraction procedures (unpublished observations). Some investigators find IS900 DNA in a proportion of patients with Crohn's disease,^{61,64,68,69,77,78,81–87} whereas others do not detect it.^{75,80,88–95}

This laboratory-to-laboratory variability in the ability to detect IS900 DNA in tissue samples from patients with Crohn's disease is important and must be addressed. In part, the variability may be due to the fact that in man MAP must primarily replicate intracellularly. As a

consequence, MAP DNA will reside in the cytoplasm of the cells of the infected host. The technical process of isolating DNA presupposes that the DNA is found within the nucleus of the cell from which it is being prepared. An initial step in DNA purification may be to remove all of the non-nuclear cellular organelles. Put simply, DNA isolation entails homogenising tissue, pelleting the nucleus, and washing away the non-nuclear cytoplasmic debris. The washed, separated, and concentrated nuclei then have their membrane disrupted, releasing their DNA.⁹⁶ However, if MAP DNA is primarily located in the cytoplasm, it will to a great extent be separated and discarded in the first isolation step: separating the nuclei from the remaining cellular constituents. Some commercial DNA/RNA isolation kits have a single-step extraction procedure. However, they assume that the DNA will be located in the nucleus. MAP DNA in the cytoplasm may be inadvertently removed with proteinaceous debris. As a consequence of these technical difficulties, specialised extraction procedures have been developed in an attempt to more reproducibly extract MAP DNA from the tissue of patients with Crohn's disease.⁸⁷

The mere presence of MAP DNA is not proof that MAP causes Crohn's disease. It is conceivable that when MAP DNA is identified in human beings the origin is from pasteurised milk from cows with Johne's disease—ie, non-viable MAP DNA was coincidentally passing through the gastrointestinal tract of the individual from whom it was isolated.

The identification of MAP RNA from patients with Crohn's disease has been called the "smoking gun" that implicates MAP as a probable cause of Crohn's disease.³⁷ This is because the isolation of MAP RNA indicates that the organism was viable at the time of isolation. Additionally, isolation of RNA is more reproducible than the isolation of DNA (unpublished observations from my laboratory). Possible explanations for this finding include the fact that the IS900 RNA is a much smaller molecule than the entire MAP genome. This fact makes isolating MAP RNA technically easier than isolating genomic MAP DNA. By contrast with the stability of eukaryotic⁹⁷ and prokaryotic⁹⁸ DNA, which may survive for centuries, RNA has a half-life that may be measured in minutes.^{99,100} The detection of the MAP RNA cannot simply be ascribed to possible environmental detritus as has been postulated with the presence of MAP DNA.

In-situ studies with IS900 probes permit the identification of MAP DNA in sheep with paucibacillary Johne's disease⁶⁶ and in human beings with Crohn's disease.^{68–70,101} An Italian study reported MAP in 73% of patients overall where 72% of granulomata and 73.9% of non-granulomatous tissue were positive.⁶⁸ A US study was positive in six of 15 Crohn's patients. Signal was detected in myofibroblasts and macrophages but not in granulomata.¹⁰¹ By contrast, an Irish study used laser capture microdissection to specifically evaluate granulomas. They reported six of 15 Crohn's granulomas were positive whereas none of the 12 control granulomas had a positive MAP signal.⁶⁹

Koch's postulates may have been met for MAP and Crohn's disease

Although *M leprae* has not fulfilled the second, third, or fourth postulate of Koch as the cause of leprosy,⁷³ because the organism cannot be grown in vitro, few would dispute the statement that leprosy is an infectious disease caused by *M leprae*.

Unlike *M leprae*, MAP can be grown in vitro, albeit with the considerable difficulty previously described.^{60,71} I suggest that Koch's four postulates¹⁰² may already have been met for MAP and Crohn's disease.¹⁰³ To accept this claim presupposes that individual publications can be combined to fulfil the postulates.

Viable MAP has been isolated and subsequently cultured by some laboratories from some patients with Crohn's disease.^{58,62,104–106} Often, the period required for the organism to grow was months to years. Given the technical difficulties of MAP culture, although not universally achievable, this finding may be considered to satisfy the first and second postulates.

Cultured human MAP^{58,107,108} has been administered orally to goats. Intestinal and mesenteric inflammation compatible with early Johne's disease was identified in one study of a single animal that was killed 5 months after inoculation.¹⁰⁷ In a more extensive study, four inoculated and four control animals were killed at 3, 5, 6, and 10 months postinoculation. The organism was identified in mesenteric lymph nodes but not in intestinal mucosa.¹⁰⁸ In other studies, no organisms were identified.⁵⁸ Human¹⁰⁷ and bovine¹⁰⁹ MAP have been inoculated intravenously or intraperitoneally in several species, resulting in liver and splenic granulomata in normal mice¹⁰⁷ and splenic isolates in immune-deficient mice.¹⁰⁹ No disease was observed in rats, guinea pigs, rabbits, or chickens,¹⁰⁷ indicating interspecies variability in susceptibility to human MAP. Unlike leprosy, where animal transmission cannot be achieved, and tuberculosis where it is, these studies may be interpreted as confirming Koch's third postulate. MAP of human^{107,108} and bovine origin has been re-isolated and recultured.^{109,110} These data may be interpreted as confirming Koch's fourth postulate.

Further studies to show that Koch's postulates are met for human MAP from Crohn's disease are indicated. Because of interspecies variation in response to MAP, the pivotal assumption is that causing Johne's disease is acceptable for establishing the second postulate. Necessary conditions are that the organism has been allowed to regain its cell wall and that sufficient time (years not months) is permitted for inoculated animals to develop Johne's disease. Unfortunately, such studies will be expensive.

Clinical trials of antibiotics to treat Crohn's disease

Published material is replete with negative studies that have attempted to treat Crohn's disease with antibiotics.^{111–125} Importantly, in all these studies the antibiotics used were ineffective against the avium species of mycobacteria in general, and MAP in particular. I suggest that studies to treat Crohn's disease with antibiotics that have no activity against



Figure 2. Tuberculoid (A) and lepromatous (B) leprosy.

M. avium and MAP are meaningless if the causative organism of Crohn's disease is MAP.

Effective therapy against *M. avium* and MAP requires the use of a macrolide antibiotic,¹²⁶ as part of a multiple-antibiotic regimen. There are now four independent publications of open-label studies of treatment of Crohn's disease with regimens containing macrolide antibiotics.^{127–130}

In a British open-label study,¹²⁷ follow-up was 7–41 months. Macrolide-containing triple antibiotic therapy was tolerated by 46 of 52 (89%) patients. 17 of 19 (91%) were successfully weaned from pretherapy steroid dependency. There was highly significant improvement in the Harvey-Bradshaw Crohn's disease activity index that was maintained at 24 months. In an American open-label study,¹²⁹ 36 patients were followed up for 4–17 months. Among Crohn's disease patients who could tolerate triple antibiotic therapy there was a sustained clinical improvement in 21 of 29 (72%). In an Australian open-label trial,¹³⁰ 12 patients were followed up for 52–54 months. Macrolide-containing triple antibiotic therapy resulted in clinical responses in eight of 12 patients. In six of 12 there was complete clinical, colonoscopic, and histological remission. Another study, apparently only ever published in abstract form, followed up 25 patients who were treated with macrolide-containing triple antibiotic therapy for about 12 months.¹²⁸ Complete remission occurred in nine of 25 (36%) patients.

Length of appropriate antibiotic therapy

In any infection, an inadequate period of appropriate antibiotic therapy may result in a transient clinical improvement that is followed by a clinical relapse. Culture-proven tuberculosis requires a minimum of 6 months' therapy. If rifampicin is not, or cannot, be used a minimum of 18 months is necessary to achieve acceptable cure rates.²⁰ Patients with AIDS and a documented *M. avium* infection require 2 years of anti-*avium* therapy,¹³¹ and indefinite therapy in the presence of ongoing immune deficiency.

In the event that Crohn's disease is shown to be due to MAP, studies will need to be done to establish the appropriate length of therapy that is required to eradicate MAP from Crohn's disease patients. Borody and colleagues found that as much as 54 months of therapy have been

necessary to induce and maintain clinical remission among patients with Crohn's disease who respond to and tolerate anti-MAP therapy.¹³⁰ There are genetically identifiable sub-populations with specific defects that compromise the affected individual's ability to cope with intracellular microbial infections.^{132–135} Such populations may require longer or indefinite therapy against MAP compared with the genetically normal population.

Comparisons among leprosy, tuberculosis, Johne's disease, and Crohn's disease

It is conceptually helpful to compare the clinical manifestations of three well-described mycobacterial diseases—leprosy, tuberculosis, and Johne's disease—with Crohn's disease. All four diseases under consideration have multiple clinical manifestations.

Possibly recognised in antiquity, leprosy has two distinct clinical manifestations (figure 2), which were designated by Hansen in 1874¹³⁶ as the “tuberculoid” and “lepromatous” forms. *M. leprae* is the organism that causes both the lepromatous and tuberculoid forms. Tuberculoid leprosy is contained, paucibacillary, and is associated with an enhanced immune response.¹³⁷ By contrast, lepromatous leprosy is uncontained, proliferative, and pluribacillary. It is the immune response of the host that establishes which form of leprosy becomes clinically manifest in a given individual, not the *M. leprae* phenotype or genotype.¹³⁷ Raised concentrations of the proinflammatory cytokine interleukin 1 β are associated with the contained tuberculoid form of leprosy.¹³⁷ Similarly, tuberculosis infection in human beings has multiple clinical manifestations. These range from the sub-clinical positive PPD test or a Ghon focus, to the aggressive pluribacillary miliary form.

It is increasingly accepted that there are two clinical manifestations of Crohn's disease (figure 3).^{138–141} They have been called “perforating” and “non-perforating” forms.¹³⁹ Perforating Crohn's disease is associated with fistulae, abscesses, and free perforation and is, in my opinion, analogous to lepromatous leprosy. By contrast, non-perforating Crohn's disease is evocative of tuberculoid leprosy. It has a more indolent course, associated with obstructive symptoms. As with lepromatous leprosy, interleukin 1 β is increased in contained (non-perforating) Crohn's disease.¹⁴²

Intestinal obstruction during antibiotic therapy for tuberculosis and Crohn's disease

Usually, during the course of a clinical trial involving medications, progression to surgery is interpreted as indicating a failure of therapy. To the contrary, I suggest that in tuberculosis as well as in Crohn's disease progression to intestinal obstruction may actually indicate a positive, albeit a paradoxical, clinical response.

One-third of patients with intestinal tuberculosis who receive appropriate anti-*M. tuberculosis* therapy may require additional surgery to relieve intestinal obstruction.¹⁴³ In patients with pre-existing intestinal strictures presumed to be due to tuberculosis on appropriate therapy, three of 39 (8%) still required surgery for obstruction.¹⁴⁴ By contrast, strictures resolved

completely on radiological examination in 16 of 21 patients (70%) who completed antituberculosis therapy and agreed to have repeat radiological evaluation.¹⁴⁴ The mechanism(s) for progression to obstruction during apparently appropriate antituberculosis therapy remain to be defined.

Empirical observations during macrolide-containing anti-MAP therapy for Crohn's disease indicate that between 8%¹³⁰ and 33%¹²⁷ of patients required surgery to relieve intestinal obstruction. This observation may simply represent an unrelated epiphenomenon. However, future studies may show that treating Crohn's disease actually results in intestinal obstruction (figure 4), which would have implications for interpretation of data from trials of macrolide-containing antibiotics in Crohn's disease.

A particularly encouraging observation from one trial of macrolide antibiotics to treat Crohn's disease is that severely diseased and immotile ileum is capable of regaining normal motility as well as normal endoscopic mucosal appearance.¹³⁰ Maintaining the patient nutritionally until the intestinal obstruction is relieved, during the course of recovery, may decrease the need for surgery to relieve intestinal obstruction.

Crohn's and tuberculosis susceptibility to genetic defects

The presence of a gene that is associated with an increased susceptibility to develop a disease does not preclude the possibility that the disease may be infectious in aetiology. A genetic defect has been identified that suggests that black people are more susceptible to tuberculosis than other races.¹⁴⁵ Similarly, an increased susceptibility to Crohn's disease, but not ulcerative colitis,¹³² is associated with a defect in the *NOD2* gene.¹³²⁻¹³⁴ It is of interest that the *NOD2* gene activates nuclear factor κ B, in response to bacterial lipopolysaccharides. Additionally, linkage disequilibrium analysis shows a common cytokine gene cluster haplotype in 5q31 that confers susceptibility to Crohn's disease.¹³⁵ Thus, genetically identifiable sub-populations may have different propensities to develop a specific disease when exposed to the identical infectious agents.

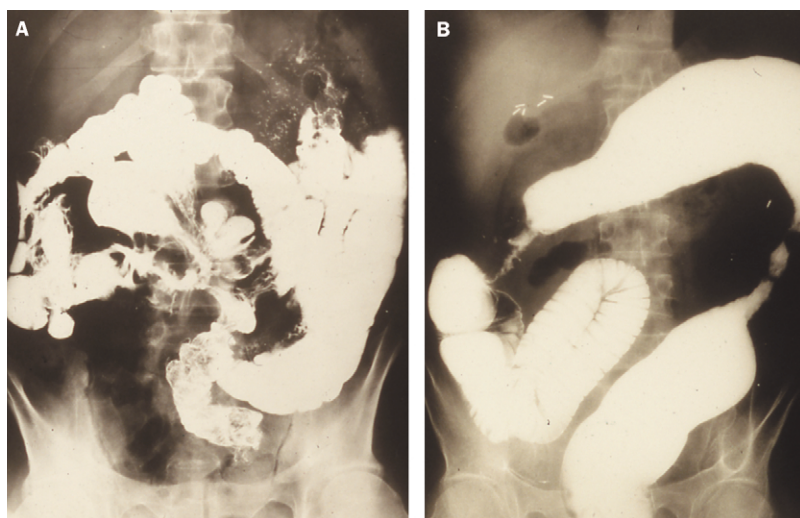


Figure 3. Gastrointestinal contrast studies from two patients with Crohn's disease. The patient on the left (A) had had multiple entero-enteric, ileo-ileal, and ileo-colic fistulae. The individual on the right (B) had non-perforating Crohn's disease. Following a previous ileo-colic resection for Crohn's disease she represented with an anastomotic and a separate descending colon stricture. From reference 141; published with permission of Elsevier Ltd.

Immune modulation and the therapy of tuberculosis and Crohn's disease

Clinical responses to immune modulation have been reported in the therapy of Crohn's disease,^{146,147} particularly the more aggressive perforating form.¹⁴⁷ In the event that Crohn's disease is eventually accepted to be infectious in aetiology, this paradox of clinical improvement of an infectious disease by immune modulation will need to be fully explained. Again it is of use to compare Crohn's disease with tuberculosis. Although tuberculosis is incontrovertibly infectious,

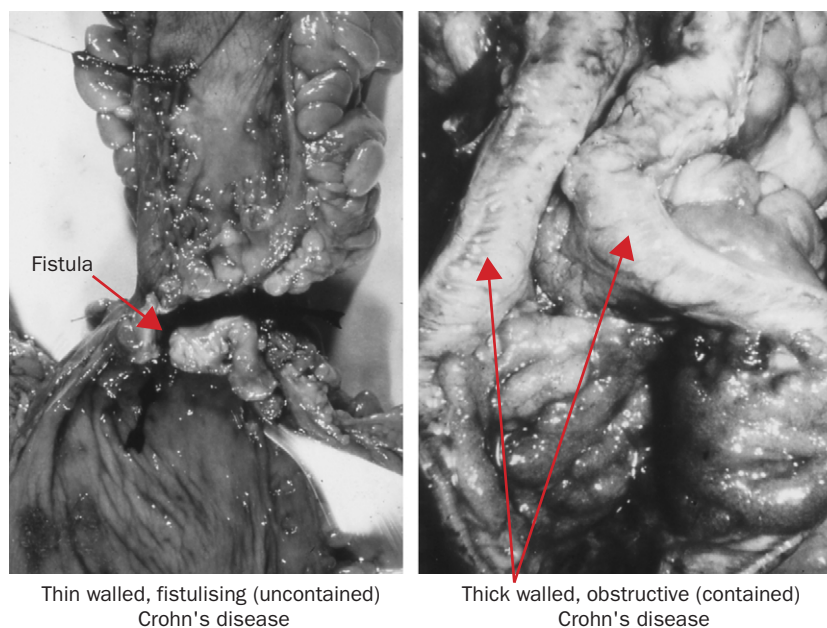


Figure 4. Gross appearance of the thin walled entero-enteric fistulas from a woman with perforating Crohn's disease (left). Note the thickened wall of a stricture in a man with chronic obstructing non-perforating Crohn's disease that required resection (right).

Search strategy and selection criteria

Searches were made using the National Library of Medicine Pubmed database. Search criteria were, individually and in combination: "Crohn", "Crohn's", "Johne's", "paratuberculosis", and "paratuberculosis and milk". English language articles and English translations of abstracts in other languages were reviewed. Websites were identified using Google and the website of the International Paratuberculosis Association (<http://www.paratuberculosis.org>).

encouraging results have been found using adjuvant cytokine therapy with interleukin 2, interferon γ , interleukin 12, and granulocyte-macrophage colony-stimulating factor to shorten treatment and address the problem of developing antibiotic-resistant tuberculosis.¹⁴⁸ There is a solitary case report of Crohn's disease developing in a patient with AIDS. Cell-wall-containing MAP were observed.³² This case may provide an opportunity to address immune mechanism that cause MAP to survive only in the cell-wall-deficient form in human beings who have normal immune mechanisms.

Conclusions and recommendations

There are increasingly compelling data that MAP, a major cause of disease in agricultural, domestic, and wild animals,

may be responsible for much more disease in humans than has been recognised. Appropriate ethical, prospective, randomised studies, are indicated among patients with Crohn's disease. These studies should use multiple antibiotics that are active against the avium species of mycobacteria. The duration of therapy needs to be determined. Individuals with *NOD2* defects may require prolonged antibiotic therapy. The importance of pre-stratifying patients into the perforating and non-perforating form of Crohn's disease has been addressed. While these studies are underway, it is probably undesirable to have viable MAP in the food supply. The USA should consider adopting the precautionary principle recently promulgated in Europe¹⁹ and require the removal of MAP from the food chain.

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Conflicts of interest

I have no conflicts of interests. The cited published studies from the Laboratory of Molecular Surgical Research of the VAMC, Bronx, NY, were funded by the Department of Veterans Affairs and patient donations. This manuscript received no funding support.

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